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14. ABSTRACT Salivary osmolality (Sosm) is a potentially useful hydration marker but may be confounded by oral artifacts. This study aimed to determine the efficacy of Sosm for detecting hypohydration and evaluate the effect of a simple mouth rinse. Eight healthy volunteers (six males and two females; age = 22 ± 7 yr, body mass = 83.7 ± 14.9 kg, height = 176.9 ± 9.2 cm) were measured for nude body mass (BM), plasma osmolality (Posm), and Sosm when euhydrated (EUH) and again when hypohydrated (HYP) by exercise-heat exposure with fluid restriction. After the initial saliva sample during HYP, a 10-s mouth rinse with 50 mL of water was provided, and saliva samples were obtained 1 min (RIN01), 15 min (RIN15), and 30 min (RIN30) after rinse. The ability of Sosm to detect HYP was compared with Posm. Results: Volunteers were hypohydrated by $-4.0\% \pm 1.2\%$ of BM (range = -2.2% to -5.3%). Sosm was elevated above EUH after hypohydration (EUH 58 ± 8 mmol•kg vs HYP 96 ± 28 mmol•kg, $P < 0.05$). Sosm baseline and change values displayed more variability than Posm based on ANOVA and regression analyses. After the oral rinse, saliva decreased in concentration (RIN01 = 61 ± 17 mmol•kg, $P < 0.05$) but returned to previous values within 15 min (RIN15 = 101 ± 25 mmol•kg, $P < 0.05$).					
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Limitations of Salivary Osmolality as a Marker of Hydration Status

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ABSTRACT

ELY, B. R., S. N. CHEUVRONT, R. W. KENEFICK, and M. N. SAWKA. Limitations of Salivary Osmolality as a Marker of Hydration Status. *Med. Sci. Sports Exerc.*, Vol. 43, No. 6, pp. 1080–1084, 2011. Salivary osmolality (S_{osm}) is a potentially useful hydration marker but may be confounded by oral artifacts. **Purpose:** This study aimed to determine the efficacy of S_{osm} for detecting hypohydration and evaluate the effect of a simple mouth rinse. **Methods:** Eight healthy volunteers (six males and two females; age = 22 ± 7 yr, body mass = 83.7 ± 14.9 kg, height = 176.9 ± 9.2 cm) were measured for nude body mass (BM), plasma osmolality (P_{osm}), and S_{osm} when euhydrated (EUH) and again when hypohydrated (HYP) by exercise–heat exposure with fluid restriction. After the initial saliva sample during HYP, a 10-s mouth rinse with 50 mL of water was provided, and saliva samples were obtained 1 min (RIN01), 15 min (RIN15), and 30 min (RIN30) after rinse. The ability of S_{osm} to detect HYP was compared with P_{osm} . **Results:** Volunteers were hypohydrated by $-4.0\% \pm 1.2\%$ of BM (range = -2.2% to -5.3%). S_{osm} was elevated above EUH after hypohydration (EUH 58 ± 8 mmol·kg $^{-1}$ vs HYP 96 ± 28 mmol·kg $^{-1}$, $P < 0.05$). S_{osm} baseline and change values displayed more variability than P_{osm} based on ANOVA and regression analyses. After the oral rinse, saliva decreased in concentration (RIN01 = 61 ± 17 mmol·kg $^{-1}$, $P < 0.05$) but returned to prerinse values within 15 min (RIN15 = 101 ± 25 mmol·kg $^{-1}$) and remained similar 30 min after (RIN30 = 103 ± 33 mmol·kg $^{-1}$). **Conclusions:** S_{osm} was remarkably altered 1 min after a brief water mouth rinse. Fifteen minutes proved an adequate recovery time, indicating that the timing of oral artifacts and saliva sample collection is critical when considering S_{osm} for hydration assessment. Given the inherent variability and profound effect of oral intake, use of S_{osm} as a marker of hydration status is dubious. **Key Words:** DEHYDRATION, FLUID INTAKE, HYPOHYDRATION, PLASMA OSMOLALITY

The noninvasive ease with which saliva samples can be obtained makes their use ideal to study physiological functions during occupational, athletic, or military tasks. Salivary osmolality (S_{osm}) has been proposed as a useful marker of hydration status because values increase when dehydration is mediated by sweat loss in controlled laboratory studies (13,20,21). Although not as widely studied as other hydration assessment markers such as blood and urine, recent research has shown that S_{osm} has marked heterogeneity (4,17) and is a poor diagnostic marker of hydration status for high-risk populations such as hospital patients with fluid–electrolyte imbalances (4). However, in lower-risk situations (e.g., occupational, sports, and military) where concern is focused on maintaining euhydration

to optimize performance in warm-hot conditions (14), S_{osm} may provide a useful measure for screening hydration status.

During athletic competitions and occupational and military activities, progressive body water deficits may occur over time while small amounts of fluid are consumed (e.g., rest breaks or water stops during a road race). Besides consuming water, other fluid and food consumption is common during athletic, occupational, and military tasks. It is unknown what effect recent fluid and food consumption might have on S_{osm} . One study presenting limited data ($n = 4$) argued that a simple water mouth rinse (insufficient to alter hydration status) seems to dilute saliva and degrade the diagnostic validity of the measures in dehydrated volunteers (4). Those findings raise questions regarding the effect of consumed beverages (water) on the magnitude, consistency, and duration of any confounding effect. Knowledge of how long S_{osm} dilution effects persist when a small amount of fluid is introduced to the oral cavity is a key to understanding the potential real-world usefulness of S_{osm} as a hydration marker in lower-risk populations.

The purposes of this study were to measure S_{osm} during euhydration (EUH) and hypohydration (HYP) and to examine the effect (magnitude and duration) of a brief mouth rinse on the recovery kinetics of S_{osm} during hypohydration. It was hypothesized on the basis of laboratory observations that S_{osm} would decline after an oral rinse with water

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but recover within 15–30 min. A rapid recovery of S_{osm} would improve its usefulness as a hydration marker for lower-risk situations. A water rinse was selected because this should have the smallest potential effect on S_{osm} compared with consumption of commercial beverages or food often taken during physical activity in warm-hot weather.

METHODS

Subjects. Eight healthy, active soldier volunteers took part in this study (six males and two females; (mean \pm SD) age = 22 ± 7 yr, body mass = 83.7 ± 14.9 kg, height = 176.9 ± 9.2 cm, body fat = $23.8\% \pm 6.5\%$). These are different subjects from those in a previous study from our laboratory examining P_{osm} (4). All volunteers passed their most recent Army Physical Fitness Test and received a general medical clearance before participation; thus, all were considered healthy and physically fit. Use of alcohol, dietary supplements, and any medication other than an oral contraceptive was prohibited. Female volunteers were not pregnant nor did any become pregnant during the study. Menstrual cycle phase and oral contraceptive use were not standardized but were considered small sources of added within-subjects biological variability (4,5,15,19) on the basis of the study question, design, and duration. For example, female sex hormones alter baseline P_{osm} to a similar degree as day-to-day variability ($\sim 4 \text{ mmol}\cdot\text{kg}^{-1}$) (16,19), whereas the P_{osm} response to dehydration and rehydration remains the same between menstrual cycle phases (15,19) and between the sexes (16). Volunteers were provided informational briefings and gave voluntary, informed written consent to participate. Investigators adhered to AR 70-25 and U.S. Army Medical Research and Materiel Command Regulation 70-25 on the use of volunteers in research. The U.S. Army Research Institute of Environmental Medicine Human Use Review Committee approved this study.

Experimental design. Each volunteer participated in all 4 d of the study protocol. During the initial 3 d, fluid intake was prescribed ($3 \text{ L}\cdot\text{d}^{-1}$), and first morning nude body mass (BM) was measured to establish baseline body mass (BM) variability (3,4) when consuming adequate fluids (9). P_{osm} , S_{osm} , U_{sg} , and BM were measured each morning on three consecutive days, so that data on biological variation could be generated using widely applied methods (6). The third day of baseline measurements (day 3) doubled as the first day of experimental testing (EUH). Volunteers were instructed to consume 1 L of premeasured water between waking and 1800 h and another 2 L of prepackaged sports drink for consumption between 1800 and 2200 h each day. Physical activity was restricted to avoid excess body water losses from sweating. Days 1 and 2 were compared to determine EUH standards for percent change in BM versus change in P_{osm} and S_{osm} . Percent change in BM was calculated by the following formula: $[(\text{day 2 BM} - \text{day 1 BM}) / \text{day 1 BM} \times 100\%]$. P_{osm} and S_{osm} were similarly compared

by subtracting day 1 values from day 2 values to determine absolute changes.

On the first day of experimental testing (day 3; EUH), volunteers reported to the laboratory at 0630 h for measures of nude BM, P_{osm} , U_{sg} , and S_{osm} . Volunteers were then free to eat and drink *ad libitum* for the remainder of the morning. On the afternoon of day 3, volunteers returned to the laboratory to perform 3–5 h of work/rest cycles (50 min of work, 10 min of rest) of treadmill ($1.56 \text{ m}\cdot\text{s}^{-1}$, 4%–7% grade) and/or cycle ergometer exercise (100–120 W) inside an environmental chamber set to 40°C , 20% relative humidity with $1 \text{ m}\cdot\text{s}^{-1}$ laminar wind flow. The purpose of the exercise-heat exposure was to increase body heat storage and induce sweating to produce hypohydration (hypertonic-hypovolemia). The range of exposure times, body sizes, and sweating rates allowed for S_{osm} responses to be examined over a range of hypohydration beyond the threshold of 2% BM loss, whereas overnight recovery after these procedures eliminated confounding factors of varying exercise modality or exposure duration. The magnitude of hypohydration (HYP) selected spanned a functionally important range of 2%–6% of BM (7,9,14). In response to exercise-heat exposure, water (sweat, urine) volume and BM losses were considered equivalent ($1 \text{ mL} = 1 \text{ g}$). The level of body water deficit was calculated from the corrected (12) change in nude BM during 24 h (from 0630 h on day 3 to 0630 h on day 4) and expressed as a percentage of day 3 EUH BM. The starting 0630 h nude BM used in the denominator of the calculation was considered EUH for an individual if it was within $\pm 1\%$ BM of his/her initial 3-d mean and confirmed EUH by measurement of P_{osm} and U_{sg} (14). Total body water (TBW) was calculated as $0.73 \times \text{lean body mass} + 0.1 \times \text{fat mass}$ because these relationships are constant throughout adult life (9). Change in TBW was calculated by dividing TBW by BM loss, with the assumption that corrected BM lost through sweating was entirely water. Body composition was assessed using sex-specific three-site skinfold measurements and estimated using appropriate formulas (10).

After exiting the environmental chamber, volunteers were provided with a small, standardized meal (450 kcal; 57% CHO, 30% fat, 13% protein, 450 mg of Na^{+}) and 200 mL of water or apple juice. No additional food or water was permitted, and volunteers were kept in supervised housing until morning. On the morning of day 4, nude BM, P_{osm} , U_{sg} , and S_{osm} measures were made once again for comparison with day 3 (EUH). The sequence of events for all measurements on days 3 (EUH) and 4 (HYP) remained consistent throughout the study. Volunteers reported each morning at 0630 h, were weighed nude after voiding a urine sample for U_{sg} analysis, and then sat quietly for 30 min before having their blood drawn and providing a saliva sample. Volunteers remained seated through blood and saliva collection to avoid potentially inducing any postural fluid shifts (8).

Analytical measures. A 3-mL sample of venous blood was collected without stasis in lithium-heparin tubes

(Sarstedt, Inc., Newton, NC) and then centrifuged at 3500g at 4°C for 10 min, and plasma was immediately separated for analysis. P_{osm} was measured in triplicate by freezing point depression on a dedicated osmometer (Fiske Micro-osmometer, Model 210, Norwood, MA) by the same technician throughout the study. First-void morning urine was provided in a sterile, inert polypropylene cup (Tyco Healthcare Group, Mansfield, MA), and a small volume was measured for specific gravity (U_{sg}) in duplicate using a refractometer (1110400A TS Meter; AO Reichert Scientific Instruments, Keene, NH). U_{sg} was selected because it is a standard euhydration assessment measure (14) and also because it is the least variable of common urine concentration measures when trying to detect change (4). Nude body mass (BM) was measured each morning on a precision platform scale (± 50 g; Model WSI-600; Mettler Toledo, Toledo, OH), which was checked daily at 25, 70, and 95 kg.

Saliva samples. A 2-mL sample of unstimulated whole saliva was collected for each volunteer on every baseline and experimental day. Volunteers produced samples during EUH and HYP by allowing saliva to collect in their mouth over time and expelling it into a small tube. After collection of the initial 2-mL saliva sample in HYP only, volunteers were given 50 mL of water and instructed to rinse their mouth for 10 s without swallowing and then spit the water back into the cup. One minute later, a second 2-mL saliva sample was collected (RIN01). Two additional samples were collected at 15 min (RIN15) and 30 min (RIN30) after the oral rinse to track S_{osm} recovery. The purpose of this rinse was to introduce a small amount of fluid to the mouth without any effect on hydration status to examine the potential confounding effect of fluid in the oral cavity in the absence of swallowing (rehydration).

Saliva samples were collected into single-use polypropylene Falcon tubes (Voigt Global Distribution, Inc., Lawrence, KS) and immediately centrifuged at 3500g at 4°C for 10 min before analysis. S_{osm} was measured immediately after centrifugation by freezing point depression by the same technician on the same osmometer (Fiske Micro-osmometer, Model 210) to minimize variability in technique. All samples were run in triplicate, and the median value was taken as final. If any of the intrasample triplicate S_{osm} measures deviated by $>3\%$, two more samples were run, and the median of five values was used. The analytical coefficient of variation (CV) for the triplicate median values was 1%.

Statistics. S_{osm} values for EUH, HYP, RIN01, RIN15, and RIN30 were compared using a one-way repeated-measures ANOVA. Tukey *post hoc* procedure was used when a significant F value occurred. Previous work using the same test method (4) showed an approximate 10% within-subjects CV for S_{osm} (~ 7 mmol·kg⁻¹) and $>50\%$ (>35 mmol·kg⁻¹) rise in S_{osm} between EUH and HYP. A sample size of eight volunteers was determined sufficient (18) to detect large (>35 mmol·kg⁻¹) changes in S_{osm} . Differences in $S_{\text{osm}} < 10$ mmol·kg⁻¹ were considered marginal *a priori*. Significance was accepted at the 95% prob-

ability level. Goodness-of-fit statistics were performed using linear regression analysis to obtain shared variance (r^2) and uncertainty (SEE) in P_{osm} and S_{osm} (y) when associated with acute measures of change (BM) in hydration state (x). Analyses were computed using GraphPad Prism 5.02 software (GraphPad Software, Inc., San Diego, CA). All data are presented as mean \pm SD.

RESULTS

Baseline variation in hydration assessment markers. The within-subjects CV_i was $<1\%$ for P_{osm} (0.3%), BM (0.6%), and U_{sg} (0.5%). The CV_i for S_{osm} was almost seven times larger (6.6%). In all cases, the between-subjects CV was larger than the CV_i , as previously reported for many biological measures (6). All values were very similar to those recently reported for hydration assessment markers using similar methods in a larger study population (4).

Hydration. All volunteers began experimental testing in a euhydrated state on the basis of meeting two or more hydration assessment criteria (14) (Table 1). All volunteers had a first-morning nude BM within 1% of their 3-d EUH average. Of eight volunteers, five had $U_{\text{sg}} < 1.020$ and two began with $P_{\text{osm}} < 290$ mmol·kg⁻¹ (14). Of eight individuals, six had starting (EUH) S_{osm} below the suggested dehydration threshold of 83 mmol·kg⁻¹ (4). No volunteer was considered overtly hypohydrated before testing on the basis of $P_{\text{osm}} < 297$ and $U_{\text{sg}} < 1.025$ (4).

After exercise-heat stress and fluid restriction procedures, volunteers were hypohydrated by $-4.0\% \pm 1.2\%$ of body mass (range = -2.2% to -5.3%), which corresponded to $-6.8\% \pm 2.0\%$ TBW (range = -4.3% to -9.4%). Table 1 provides body mass, TBW, and hydration marker values when euhydrated and hypohydrated. P_{osm} , U_{sg} , and S_{osm} all increased in response to fluid losses as expected. Of eight volunteers in HYP, five surpassed the proposed S_{osm} diagnostic threshold for hypohydration of 83 mmol·kg⁻¹ (4), whereas seven surpassed the proposed threshold for P_{osm} hypohydration of 297 mmol·kg⁻¹ (4).

Figure 1 plots the individual percent change in BM and TBW versus P_{osm} (A) and S_{osm} (B) during HYP. Shared variance was approximately 75% for associations between change in absolute P_{osm} and change in BM or TBW ($r = -0.85$ to 0.87), whereas shared variance was approximately 50% for S_{osm} ($r = -0.71$ to 0.73) when associated with change in BM or TBW. Similar trends were seen when percent changes in BM and TBW were compared with the

TABLE 1. BM, TBW, P_{osm} , U_{sg} , and S_{osm} during EUH and HYP.

	EUH	HYP
BM (kg)	83.8 \pm 14.8 (69.5–109.8)	80.4 \pm 13.7 (67.2–104.5)
TBW (kg)	48.8 \pm 10.0 (36.2–63.7)	45.3 \pm 8.9 (34.7–58.4)
P_{osm} (mmol·kg ⁻¹)	291 \pm 3 (285–293)	303 \pm 7 (294–315)
U_{sg}	1.018 \pm 0.005 (1.010–1.024)	1.028 \pm 0.003 (1.022–1.032)
S_{osm} (mmol·kg ⁻¹)	58 \pm 8 (50–71)	96 \pm 28 (70–154)

Values displayed are mean \pm SD (range).

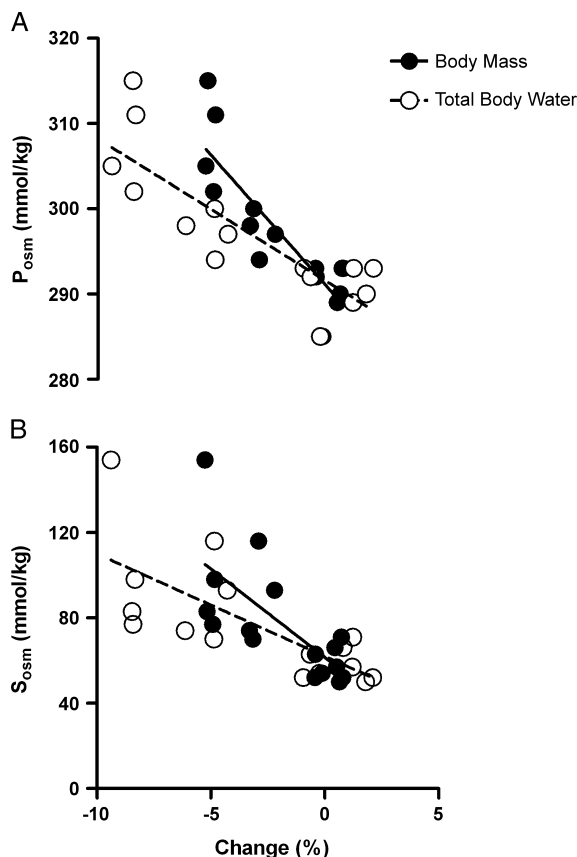


FIGURE 1— P_{osc} (A) and S_{osc} (B) values during EUH and HYP plotted against percent change in body mass and TBW, with best-fit linear regression for each.

change in P_{osc} ($r = -0.87$) and S_{osc} ($r = -0.71$) from EUH to HYP. The SEE for saliva ($19\text{--}20\text{ mmol}\cdot\text{kg}^{-1}$) was four to five times larger than that for plasma ($4\text{--}4.6\text{ mmol}\cdot\text{kg}^{-1}$) whether comparing absolute or change values.

Salivary osmolality recovery dynamics. Figure 2 presents the influence of a water rinse on S_{osc} values during a 30-min period. After the oral rinse, S_{osc} values decreased ($P < 0.05$) to EUH levels, despite no fluid intake or change in hydration status. Only one S_{osc} value remained over the proposed diagnostic threshold for hypohydration of $83\text{ mmol}\cdot\text{kg}^{-1}$ (4) after the oral rinse ($RIN01 = 61 \pm 17\text{ mmol}\cdot\text{kg}^{-1}$). Fifteen minutes later, S_{osc} values had returned to the prerinse HYP values, with six of eight values surpassing the proposed diagnostic threshold for hypohydration (4). Values were nearly identical at 15 min ($RIN15 = 101 \pm 25\text{ mmol}\cdot\text{kg}^{-1}$) and 30 min ($RIN30 = 103 \pm 33\text{ mmol}\cdot\text{kg}^{-1}$) after rinse.

DISCUSSION

Measuring and maintaining hydration state has important implications for health and performance, but a simple, noninvasive, and accurate measure of hydration status remains elusive (1). S_{osc} has been proposed as a marker of

hydration status because of the ease of collection in real-world scenarios. However, S_{osc} has only been tested in controlled laboratory conditions, which are unlikely to mimic real-life fluid consumption and eating patterns typically encountered during actual occupational, sports, or military medicine use. The present study carefully measured hydration status and S_{osc} and experimentally examined day-to-day variability and effect of mouth rinse on S_{osc} . The collected information is important in determining whether S_{osc} might be used as a hydration marker in real-life, low-risk situations. The primary findings of this investigation were as follows: 1) S_{osc} enabled distinction between EUH and HYP conditions in most well-controlled cases, 2) S_{osc} demonstrated considerable variability in day-to-day measurement and in magnitude of response to HYP, 3) S_{osc} is easily confounded after a brief oral rinse, but 4) S_{osc} values recovered within 15 min of rinsing.

S_{osc} increased in all eight volunteers in response to fluid losses of 2.2%–5.3% body mass (4.3%–9.4% body water loss), which matches previous studies where hypohydration in the range of 2%–7% body mass reliably increased S_{osc} (4,20,21). However, shared variance was less, and the SEE was much larger between the level of dehydration and either the absolute S_{osc} (Fig. 1B) or its change compared with P_{osc} (Fig. 1A). This is likely explained by large variability in S_{osc} between subjects (4,17). Thus, S_{osc} is an imprecise discriminator between levels of hypohydration (mild ($\sim 2\%$ BM loss) versus moderate to severe ($\geq 4\%$ BM loss)) when examined in cross section. When serial S_{osc} samples were taken from individuals undergoing progressive hypohydration during a single test session, a much stronger relationship has been reported (e.g., $r = 0.94$) (18). However, day-to-day variability in S_{osc} was nearly 10 times larger in this and other studies (4) than P_{osc} , BM, or U_{sg} . A recent work (4) also showed that a significant non-uniformity of variances within subjects would make daily S_{osc} change monitoring of questionable diagnostic value.

S_{osc} was confounded by a brief oral rinse with water 1 min after rinse, resulting in values no different from

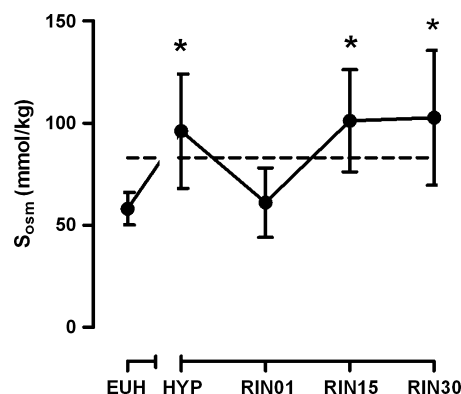


FIGURE 2— S_{osc} values (mean \pm SD) during EUH, HYP, 1 min (RIN01), 15 min (RIN15), and 30 min (RIN30) after oral rinse. Dotted line denotes the proposed dehydration diagnostic threshold of $83\text{ mmol}\cdot\text{kg}^{-1}$ (4). *Significantly different from EUH and RIN01.

EUH and well below the threshold for HYP (4), despite no change in hydration status. However, most values recovered within 15 min of rinsing (Fig. 2), indicating that 15 min is an adequate time standard for field use between water consumption and saliva sampling. Sports drinks, food, or gum may contain numerous factors (carbohydrate, Na^+ , K^+ , etc.) that are likely to additionally confound S_{osm} results. As a result, the practical usefulness of S_{osm} may be limited in a real-world scenario where athletes or soldiers are consuming fluids, eating, chewing gum or tobacco, and smoking. The effect of other oral intake has not yet been examined, although all of these confounders are likely to affect S_{osm} outcomes.

Another potential consideration in addition to oral intake is the effect of aerobic and anaerobic exercises on S_{osm} . Salivary Na^+ , K^+ , and protein concentrations have all been found to increase after a bout of exercise (2,11). Changes in blood flow to the salivary glands and catecholamine-induced shifts of water from circulation can also affect S_{osm} without a meaningful change in hydration status. In athletic events, labor, and military operations, physical activity may further confound S_{osm} and must also be considered in examining the applicability of S_{osm} for hydration

assessment in real-world scenarios. Future research may examine additional possible confounding scenarios such as recent exercise, carbohydrate-electrolyte beverage consumption, and even the ability of S_{osm} to distinguish between euhydration and hyperhydration or hyponatremia, a serious risk in endurance sporting events.

In conclusion, a brief oral rinse easily confounds S_{osm} measurement during hypohydration, indicating that S_{osm} is of limited use for hydration assessment when fluids are regularly being consumed. Although S_{osm} values recover within 15 min of oral intake, timing of fluid intake is paramount in obtaining a valid measure of hydration status through S_{osm} . Given the inherent variability and profound effect of oral intake on S_{osm} , its use as a marker of hydration status is dubious.

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The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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